IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION OF

FODOR et al.

Appln. No. 09/776,768 Group Art Unit: 1656

Filed: February 6, 2001 Examiner: to be assigned

FOR: IDENTIFYING A BASE IN A NUCLEIC ACID (as amended) Atty. Dkt.: 266873

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October 29, 2001

AMENDMENT TO COMPLY WITH SEQUENCE LISTING RULES

Hon. Commissioner of Patents
Washington, D.C. 20231

Sir:

In response to the Notice of Incomplete Reply dated October 4, 2001, Applicants submit the following amendments to comply with Sequence Listing Rules.

Amendments

Please enter the enclosed paper copy of the Sequence Listing after the claims and abstract of the application.

Please amend page 2 of the application, lines 21-40, to read as follows:

> When the nucleic acid probes are of a length shorter than the target, one can employ a reconstruction technique to determine the sequence of the larger target based on affinity data from the shorter probes. See U.S. Patent No. 5,202,231 to Drmanac et al., and PCT patent Publication No. 89/10977 to Southern. One technique for overcoming this difficulty has been termed sequencing by hybridization or SBH. For example, assume that a 12-mer target DNA 5′-AGCCTAGCTGAA (SEQ ID NO:1) is mixed with an array of all octanucleotide probes. If the

target binds only to those probes having an exactly complementary nucleotide sequence, only five of the 65,536 octamer probes (3′-TCGGATCG, CGGATCGA, GGATCGAC, GATCGACT, and ATCGACTT) will hybridize to the target. Alignment of the overlapping sequences from the hybridizing probes reconstructs the complement of the original 12-mer target:

A1 Cont'd

TCGGATCG
CGGATCGA
GGATCGAC
GATCGACT
ATCGACTT
TCGGATCGACTT (SEQ ID NO:2)

Please amend page 17 of the application , lines 24-37, to read as follows:

An octanucleotide array of MenPoc-dG and MenPoc-dT was synthesized. The format of the synthesis was similar to that for the $(A+T)^8$ array, discussed above, and resulted in 256 octanucleotides of G and T in replicates of four (1024 total). After final deprotection and attachment to a temperature-controlled (15°C) hybridization chamber, the probe array was incubated with 1 nM 5′-AACCCAAACCC-fluorescein (SEQ ID NO:3- fluorescein) target and scanned. The resulting image is given in Fig. 10. Four distinct but overlapping, perfectly complementary octanucleotide hybridizations are expected: 3′-TTGGGTTT, TGGGTTTG, GGGTTTGG, and GGTTTGGG. As shown herein, the moderate stability of probe/target complexes with single base pair mismatches generates families of probes with moderate signals. A cursory inspection of the many intense features of Fig. 10 revealed a complex pattern.

A2

Please amend the application on page 25, line 33 - page 26, line 19, to read as follows:

A (G+T)^8 array was prepared and incubated with 1 nM 5′-AACCCAACCCC-fluorescein (SEQ ID NO:4-fluorescein) (representing a mutant sequence when compared to 5′-AACCCAAACCC (SEQ ID NO:3)) and scanned to test whether the sequence was "wild" or "mutant." The resulting image is given in Fig. 16. Four overlapping, exactly complementary octanucleotide

A3

probe/target hybridizations are expected if one is assuming the target should be 5′-AACCCAAACCC (SEQ ID NO:3) with probes: S-3′-TTGGGTTG, TGGGTTGG, GGGTTGGG, and GGTTGGGG. The results demonstrated that the effect of a single base change is quite dramatic, especially in the number and identity of the different mismatched probe/target complexes that form on the array. If one assumes the target nucleic acid generating the signal in Fig. 16 is 5′-AACCCAAACCC (SEQ ID NO:3) (i.e., the wild-type) then the mismatch profiles for the complementary probe S-3′-TTGGGTTT are shown in Fig. 17A. The mismatch profile does not have the expected shape, and the probe/target complex has a low fluorescence intensity. The strong peak corresponding to a mismatch in position 8 indicates that the "correct" base in this position in the target is probably an A, because only A and C are found in the target in this experiment. Mismatch position 6 also shows a small peak. By contrast, a similar plot using the probe sequence S-3′-TTGGGTTG probe sequence as a core yielded the "smile" shape and high fluorescence intensity. In Fig. 17B the same profile for the next 8-mer probe is shown. The peaks have shifted one position to the left, again confirming that the sequence varies from wild-type at position 8 in the target. These correspond to the same positions in the original 11-mer target fragment. These data predict that there is a single base change in position 8 of the target, as compared to the wild-type.

All of the mismatch probe profiles corresponding to the assumed fragment 5′-AACCCAACCCC (SEQ ID NO:4) are shown in Fig. 17C. One observes the mutant position "moving" down the sequence. Finally, in Fig. 17D the mismatch plots are shown corresponding to the four probes that complement 5′-AACCCAACCCC (SEQ ID NO:4), with the expected smile characteristics.